

Comm.

Dr. Cattell
Dr. Jacobson
Dr. Sommers

CARDIOVASCULAR

#310R12

(Renewed annually
since 7/1/62)

#CF #91 - 7/1/55

#CF #270-7/1/60-7/1/61

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A., INC.

110 EAST 58TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

FEB 1 1972

Application For Renewal of Research Grant

(Use extra pages as needed)

First Renewal ☐

Second Renewal ☐

Date: 1/31/73

1. Principal Investigator (give title and degrees): Richard J. Bing, M.D.
Professor of Medicine, University of Southern California
Director of Cardiology and Intramural Medicine,
Huntington Memorial Hospital
Visiting Associate in Biomedical Engineering,
California Institute of Technology
2. Institution & address:

Huntington Memorial Hospital
100 Congress Street
Pasadena, California 91105

3. Department(s) where research will be done or collaboration provided:

Huntington Institute of Applied Medical Research
Huntington Memorial Hospital

4. Short title of study:

Carbon Monoxide and Coronary Atherosclerosis

5. Proposed renewal date: July 1, 1973

6. How results to date have changed earlier specific research aims:

The research aims have expanded to include carbon monoxide. No change in the general specific aims have resulted from our findings.

7. How results to date have changed earlier working hypothesis:

Our working hypothesis has been to study human coronary atherosclerosis by using human coronary arteries. Our results are included in our progress report.

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8. Any additional facilities now required? Describe briefly.

(3)

None

9. Any changes in personnel? Append biographical sketches of new key professional personnel:

The biochemical execution of this work is under the supervision of Dr. James C. Mao, whose curriculum vitae is enclosed. The overall direction of this project remains with Dr. Richard Bing, the principal investigator.

10. Append outline of experimental protocol for ensuing year. See enclosed.

11. List publications or papers in press resulting from this or closely related work. (append reprints or manuscripts not previously sent).

See enclosed.

12. Summary progress report (append in standard form as separate document, unless recently submitted).

See enclosed.

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13. Budget for the coming year:

A. Salaries (give names or state "to be recruited")

% time

Amount

Professional (give % time of investigator(s)
even if no salary requested)

James C. Mao, Ph.D.

90

Fringe Benefits

H. Tillmanns, M.D.

30

J. M. Fauvel, M.D.

30

R. J. Bing, M.D.

70

Technical

E. Larsen, B.S.

100

Fringe Benefits

K. Seeler, M.S.

70

H. Hansen, B.A.

30

Sub-Total for A

B. Consumable supplies (by major categories)

Isotopes, reagents, enzymes,
catheters, syringes, general
chemicals

9,000

Sub-Total for B

9,000

C. Other expenses (itemize)

Expenses in obtaining arteries and veins
and for histological examinations

1,000

Sub-Total for C

1,000

Running Total of A + B + C \$33,298

D. Permanent equipment (itemize)

Lindbergh pump

600

Sub-Total for D

600

E. Indirect costs (15% of A+B+C)

E

4,995

Total request

\$38,893

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1. List financial support from all sources, including own institution, for this and related research projects.

| Title of Project | Source (give grant numbers) | Amount | Inclusive Dates |
|--|--|----------|---------------------|
| "The Direct Effect of Nicotine and Catecholamines and Their Agonists and Antagonists on the Coronary Microcirculation" | American Medical Association Education and Research Foundation | \$33,312 | 11/1/72 to 10/30/73 |
| "The Effect of Alcohol on the Heart" | National Institutes of Health, MH21052-02 | \$38,700 | 1/1/73 to 12/31/73 |

| Title of Project | Source (give grant numbers) | Amount | Inclusive Dates |
|------------------|--------------------------------|--------|-----------------|
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Telephone (213) 796-0371 S10
Area Code Number Extension

Huntington Memorial Hospital
Pasadena, California 91105

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APPLICATION FOR RENEWAL OF RESEARCH GRANT

Richard J. Bing, M.D.
Huntington Memorial Hospital
100 Congress Street
Pasadena, California 91105

Project Title: The Effect of Carbon Monoxide on Atherosclerosis
of Coronary Arteries and Saphenous Veins of Man
(In Vitro Studies)

Background of Proposed Work

As described in our progress report, we have studied the lipid metabolism in perfused human atherosclerotic and nonatherosclerotic coronary arteries and saphenous veins. In atherosclerotic coronary arteries, it could be established that these vessels cannot synthesize cholesterol from acetate, that only very limited synthesis of cholesterol esters from acetate occurs, that free cholesterol is taken up by the artery, and, finally, that nicotine has a very slight effect on lipid synthesis from acetate but does not influence free cholesterol uptake.

Our experiments then were extended to include nonatherosclerotic human coronary arteries and saphenous veins. The arteries were obtained within five hours following death of the patient and the saphenous veins at the time of operation for aortocoronary bypass.

The results obtained on nonatherosclerotic human coronary arteries were quite similar to those obtained on the diseased vessels. Consequently, human coronary arteries without evidence of atherosclerosis did not differ from the atherosclerotic vessels in their inability to synthesize cholesterol from acetates;

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coronary arteries with and without atherosclerotic lesions take up cholesterol from the perfusion fluid to the same degree; human saphenous veins perfused at relatively low pressure (45/35 mmHg) did not differ from atherosclerotic and normal coronary arteries in their ability to synthesize lipids; the uptake of cholesterol was less than that of coronary arteries or saphenous veins perfused at arterial pressure of 130/100 mmHg; human saphenous veins perfused at arterial pressure take up cholesterol from the perfusion fluid equal to that taken up by the coronary arteries; and, in saphenous veins perfused at high pressure, synthesis of lipids from acetate equals that in saphenous veins perfused at lower pressure of nonatherosclerotic coronary arteries. In these experiments, we could demonstrate that nicotine failed to influence either synthesis of lipids or uptake of cholesterol in veins perfused at 45/35 mmHg.

From these two studies we concluded that nicotine has no appreciable influence on either synthesis of lipids in human coronary arteries or veins or on the uptake of cholesterol and cholesterol esters by these vessels.

It was of further interest that there is no synthesis of cholesterol in either coronary arteries or veins, but that cholesterol uptake in arteries and veins is considerable, particularly if the veins are perfused at arterial pressures. This latter point may be of importance in assessing the future of aortocoronary bypass, which is now commonly practiced on patients with coronary artery disease.

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Proposed Work

The aim of our proposed investigation is a study of the effect of carbon monoxide on lipid metabolism in perfused coronary arteries and saphenous veins.

The effect of carbon monoxide on the production of atherosclerosis has been discussed by a number of investigators. It has been questionable, however, whether this effect is due to hypoxia or to a specific effect of carbon monoxide. Astrup (Journal of Atherosclerosis Research, 7:343, 1967) exposed an experimental series of rabbits to small concentrations of carbon monoxide and a control series to room air. He observed (1) that the degree of visible aortic atheromatosis and the content of total cholesterol in the aortic tissue were significantly higher in the rabbits exposed to carbon monoxide than in the control, and (2) that, in about three fourths of the carbon-monoxide-exposed rabbits, the hearts also displayed pathologic changes. Microscopic examination also supported these findings; it was found that the underlying biochemical and physiological mechanisms may be explained by tissue hypoxia due to a carbon-monoxide-induced displacement of the oxygen dissociation curve to the left, in combination with a decreased activity of certain enzymes inhibited by carbon monoxide.

In another study, the same group of investigators has determined the cholesterol level of aortic tissue in the control group and in animals exposed to carbon monoxide and found that

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the amount of this lipid was about five times as high as that in the control group. In line with this observation were macroscopic findings which demonstrated that the vascular wall showed various amounts of lipid deposits. This lipid material was predominantly seen in the intima, which appeared markedly thickened. The coronary vessels of all dimensions showed similar changes. Astrup and coworkers believe that these alterations are due to a more general change of the vessel wall. They are of the opinion that CO increases capillary permeability, as first postulated by Kjeldsen and Giese. This hypothesis, that the alterations in the vascular wall induced by carbon monoxide are due to an increase in vascular permeability, was confirmed by Andersen and his coworkers (Andersen, et al., Scandinavian Journal for Clinical and Laboratory Investigation, 22:39, 1968). They tested the permeability of these vessels by means of penetration of labelled albumin (^{131}I -albumin). Whether the studies of Astrup and associates are valid is as yet doubtful. Astrup and coworkers (Atherosclerosis) found changes similar to those which are produced by carbon monoxide in animals exposed to hypoxia.

In this country, most of the studies on the effect of carbon monoxide on cardiovascular disease are based on the work of Aronow. This investigator found in 1971 (Circulation, 54:782, 1971) that, following the smoking of non-nicotine cigarettes, the mean carboxyhemoglobin level rose from 1.6 to 7.8%.

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Smoking significantly decreased the mean exercise time from the onset of exercise until the onset of angina by a considerable range. One particular finding is of interest, namely that smoking non-nicotine cigarettes increased the carboxyhemoglobin level, decreasing the rate of oxygen-carrying capacity to the myocardium; as a result angina develops sooner with less cardiac work. This particular statement is surprising because, in another study published in the Annals of Internal Medicine, 74:697, 1971, the same author analyzes the effects of smoking on the cardiovascular system. He concluded that the main hemodynamic alterations are due to the effect of nicotine and not to that of carbon monoxide. Finally, in a recent paper (Annals of Internal Medicine, 77:669, 1972) Aronow and coworkers found that freeway traveling markedly increased carbon monoxide concentration in the blood and diminished exercise performance until angina appeared. He also observed ischemic ST-segment depression in three of his ten patients while breathing freeway air. Roughly, the arterial carboxyhemoglobin level increased from about .8 to about 7.5%.

The main conclusions drawn from these studies in the literature are as follows: (1) The effect of carbon monoxide on atherosclerosis may be a nonspecific effect similar to that induced by hypoxia. This has been demonstrated by increased permeability under both sets of experimental conditions.

(2) The hemodynamic effects of smoking are in all likelihood not the result of carbon monoxide but of nicotine.

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These changes also suggest that carbon monoxide has an effect on permeability of the vascular wall. Chance has shown that there are two different carbon monoxide targets, both of which involve changes in hematin-iron linkage. The first target exists at the blood level, where the reaction of carbon monoxide with hemoglobin creates carboxyhemoglobin, which is inactive in oxygen transport; the second exists at the cellular level where the hematin-iron atoms of 3A -cytochrome are liganded by CO, thereby losing their reactivity toward oxygen. The former target has been the subject of extensive investigations by many physiologists; the latter remains the domain of the biochemist. It is likely that carbon monoxide makes itself felt in both directions, (a) through the effect of the dissociation curve, and (b) through the mitochondrial-electron transfer chain. Chance explains the latter as a loss of the ability of the matrix of enzymes involved in membrane-bound electron flow to be activated effectively to establish redox patterns of optimal effectiveness by remaining uninhibited cytochrome molecules, thus enabling the flow of oxidizing equivalents to spread through the totality of the membrane components. He emphasizes the extreme sensitivity of mitochondria in the uncoupled or metabolically active state to small concentration of carbon monoxide.

Method of Procedures

Human coronary arteries (atherosclerotic and nonatherosclerotic) and saphenous veins will be perfused as described in our previous

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applications (see progress report). In brief, these arteries will be perfused in a modified Carrel-Lindbergh pump at pulsatile pressure. Arteries will be perfused at pressures of 120/80 mmHg, and veins will be perfused at a pressure below 35 mmHg. In contrast to previous work, where the perfusion fluid consisted of human plasma, we plan to use diluted fresh human blood. The reason for this change in technique is that alterations in oxygen dissociation curve would of course not occur in the absence of the respiratory pigment. On the other hand, in later experiments, it may be possible to perfuse the vessels with plasma alone in order to test the hypothesis of Chance on the effect of carbon monoxide on the mitochondrial respiratory chain alone.

As previously stated, sterile techniques will be used during the preparation and perfusion, and perfusion will be carried out over a period of 4 hours at 37°C. The gas which will drive the fluid through the artery and which will come into equilibrium with the perfusion fluid will consist of 5% CO, 5% CO₂, 20% O₂, and 70% N₂O. In the control experiments, CO will be omitted. It is assumed that this will give an approximate concentration in the perfusion fluid of carbon monoxide of about 15%. To the perfusion fluid will be added 2-¹⁴C-sodium acetate and cholesterol-1,2-³H. Cholesterol will be brought into solution by sonication, as described in previous reports. It has been shown that the tritium radioactivity is

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located primarily in the alpha-2-lipoprotein and in the beta-lipoprotein fraction. Lipids will be analyzed in the perfusion fluid prior to and following perfusion. Analysis will be carried out on the whole blood vessel, and the extraction of the lipids will be carried out according to the method of Folch. Separation of the lipids will be accomplished by means of thin-layer chromatography on silica gel according to the method of Freeman and West. Radioactivity of the eluate will be determined in a scintillation vial and counted in a tricarb liquid scintillation spectrometer. The method of Zak will be used for the determination of cholesterol in plasma extract in the eluate. Phospholipids are analyzed according to the method of Lowry modified by Wagner. The blood vessels will then be analyzed for lipid synthesis as well as for cholesterol and cholesterol ester uptake, and statistical analysis will be used to see whether any significant differences exist between blood vessels perfused under normal conditions and those with perfusion fluid containing carbon monoxide hemoglobin.

Significance of This Work

The significance of this work is twofold. First, it will permit further investigations on the mechanisms of atherosclerosis in human vessels. Since most of the studies concerning the mechanism of coronary atherosclerosis have been performed on animals, the use of human blood vessels constitutes a certain advantage.

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Second, the question of the effect of carbon monoxide on the mechanism of atherosclerosis has so far been unsettled. Studies have either been carried out on animals, or they have only been restricted to the synthesis of cholesterol in animal blood vessels. Therefore, although the main thrust of this project is directed toward the effect of carbon monoxide, it has more general implications for the overall field of coronary atherosclerosis.

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CURRICULUM VITAE

James C. Mao, Ph.D.

PERSONAL
REDACTED
REDACTED

Birthplace:

REDACTED

EDUCATION

B.S., National Taiwan University

M.S., North Dakota State University

Ph.D., North Dakota State University

Postdoctoral fellow (1971-1972) under Dr. Rubin Bressler,
Department of Pharmacology, College of Medicine, University of
Arizona, Tucson, Arizona

PUBLICATIONS

1. Huxtable, R., Mao, J., Hayes, J. S., Bressler, R., and Picchioni, A. L. 1972. Biochemical changes in the sarcoplasmic reticulum of muscle from androgenically epileptic rats upon seizure. (Submitted to the Journal of Clinical Investigation).
2. Hashimoto, H., Sarma, J. S. M., Mao, J., Larsen, E., Tillmanns, H., and Bing, R. J. 1973. Lipid metabolism in perfused human nonatherosclerotic coronary arteries and saphenous veins. (Submitted to Atherosclerosis).
3. Pachinger, O. M., Mao, J., Hashimoto, H., Hellberg, K. D., Tillmanns, H., and Bing, R. J. 1973. The effect of prolonged administration of ethanol on cardiac metabolism and performance in the dog. (Submitted to the Journal of Clinical Investigation).

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